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# Histone purification from *Saccharomyces cerevisiae*

Frederic Jourquin and Vincent Géli

Cancer Research Center of Marseille (CRCM), U1068 Inserm, UMR7258 CNRS, Aix Marseille University (AMU), Institut Paoli-Calmettes. Marseille, F-13009. France.

## Summary

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The nucleosome structure consists of a histone octamer made by a tetramer of H3-H4 histones and two dimers of H2A-H2B. Nucleosomes undergo extensive post-translational modifications that regulate nucleosome interactions, position, and stability. We describe a protocol allowing the robust purification of histones from the yeast *Saccharomyces cerevisiae*. This method appears to be suitable to quantitatively analyse specific posttranslational histone modifications.

**Key words** : Histones, chromatin, purification, budding yeast

## 1. Introduction

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This protocol to purify yeast histones by acid extraction of isolated chromatin is adapted from Dehe et al (2005) and Poveda et al (2004). The protocol is written for an amount of wet yeast cells of 100 mg. It can be adapted for larger amount of wet cells.

## 2. Materials

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All solutions are prepared using ultrapure water (prepared from deionized water to reach the 18,2M  $\Omega$  cm at 25°C)

1. YPD:xxx
2. Buffer 1 (extraction buffer pH=7,5) : 1 M sorbitol, 50mM Tris-HCl pH=7,5, 5 mM MgCl<sub>2</sub> (keep at -20°C)
3. Buffer 2 (extraction buffer pH=6) : 1M sorbitol, 50 mM MES pH=6, 5 mM MgCl<sub>2</sub> (buffered with KOH 2M and keep at -20°C)
4. Buffer 3 (lysis buffer K) : 50 mM MES pH=6, 75 mM KCl, 0,5 mM CaCl<sub>2</sub>, 0,1% NP-40 (keep at -20°C)
5. Buffer 4 (lysis buffer HS) : 10 mM MES pH=6, 430 mM NaCl (keep at 4°C)
6. PI (Protease Inhibitors). For a 20 ml solution: 400  $\mu$ l of PI complete mini EDTA-Free (Roche), 20  $\mu$ l of 20mM 3-4 Dichloroisocoumarin serine protease inhibitor (Sigma Aldrich), 40  $\mu$ l of PMSF 0,5 M (prepared in DMSO solution)

7. Zymolyase (R) 20T from *Arthro bacter luteus*
8. 2-mercaptoethanol (14.3M)
9. IGEPAL® CA-630 detergent (Nonidet P-40 substitute)
10. 0.25M HCl
11. Acetone
12. Acidified acetone: 120mM HCl in acetone
13. loading buffer (2X):xxx
14. Coomassie staining : 50% ethanol, 7% acid acetic, 0,5g/L Coomassie brilliant blue R250. Complete to 1L with distilled water. Store at room temperature.
15. Coomassie Destaining : 20% ethanol ; 7% acid acetic. Store at room temperature.

### 3. Methods

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#### 3.1 Cell sample preparation

1. Inoculate 100 ml YPD with a wild type strain (W303) (See **note 1**) to an OD<sub>600</sub> below 0,1. Grow the culture at 30°C
2. Harvest the cells at OD<sub>600</sub> = 0,8 and centrifuge the culture for 5 min at 3000 rpm
3. Wash the cell pellet twice in water
4. Wash the cell pellet with buffer 1
5. Weight the cellular pellet
6. Prepare samples of 100 mg of wet cells in 2 ml eppendorf tubes
7. Wash the cell pellet with buffer 1
8. Discard the supernatant (you can stop the protocol at this step by freezing the pellets in liquid nitrogen and storage at -80° C.)

#### 3.2 Spheroplast preparation

1. Cell pellets of 100 mg are resuspended in 1 ml of buffer 1. Add 5,4 µl of 2-mercaptoethanol (14,3 M). Incubate in ice during 10 min
2. Centrifuge at 4°C for 5 min at 3000 rpm and discard the supernatant
3. Resuspend the pellet in 1 ml of buffer 1 (without 2-mercaptoethanol). Add 600 µg of zymolyase 20T (see **note 2**)
4. Incubate with gentle agitation for 30 min at 35°C
5. Add directly in the tube 700 µl of buffer 2 containing a mixture of protease inhibitors (PI) (see **Note 3**)
6. Centrifuge 5 min at 4°C at 3500rpm and discard the supernatant (to discard any traces of zymolyase)

7. *Optional: add 2ml of buffer 2 + PI without resuspension of the pellet and centrifuge at 4°C for 5 min at 3500rpm. Discard the supernatant*

### 3.3 Extraction

All the following steps are to be performed on ice, unless otherwise indicated.

1. Resuspend the pellet in 700 µl of buffer 3+PI. Incubate for 5min on ice
2. Centrifuge for 5min at 11000 rpm at 4°C and discard the supernatant
3. Resuspend the pellet in 700 µl of buffer 4. Add IGEPAL® CA-630 detergent to a final concentration of 0,5% and PI (*see Note 4*). Let in ice for 5 min.
4. Centrifuge at 4°C for 5 min at 12000 rpm and discard the supernatant
5. Resuspend the pellet in 700 µl of buffer 4+PI and let on ice for 5 min.
6. Centrifuge at 4°C for 5min at 13000rpm, discard the supernatant
7. Add 120 µl of 0,25M HCl
8. Place a small magnetic barrel in the tube and agitate for 2h (minimum) at 4°C (a rotor wheel can be also used).
9. Centrifuge at 4°C for 15 min at 12000 rpm
10. Transfer the supernatant in a 15 ml Falcon tube
11. Add 8 volumes of acetone for 1 volume of supernatant
12. Mix by inversion and place the tube overnight at -20° C
13. Centrifuge at room temperature for 10 min at 3500 rpm and discard the supernatant
14. Resuspend the pellet with 400 µl of acidified acetone and transfer the resuspended pellet in an 1.5ml tube.
15. Centrifuge at RT for 5 min at 10000 rpm. You should see a white visible pellet.
16. Discard the supernatant and add 1ml of acetone and carefully slowly vortex
17. Centrifuge at RT for 5min at 10000 rpm and discard the supernatant
18. Dry the pellet with a speed-vac (no more of 20 min) or let it dry at 37°C (*see note 5*)
19. Resuspend the pellet with 10 µl of distilled water.
20. Add 5µl of loading buffer (2X) to 5µl of purified histones. The remaining histone preparation can be kept at -20°C.
21. Denature for 5 min at 100°C.
22. Load 2 µl on a 16% SDS-PAGE (*see note 6*) and run at 100V for 3h

### 3.4 Coomassie Staining

1. Stain the acrylamide gel in the Coomassie staining solution for 30 min
2. Destain for one hour in the Coomassie destaining solution

**(Figure 1)**

## 4. Notes

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1. W303 MATa {*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*}

2. Do not add an excess of zymolyase, it may result in the clipping of the N-terminal tail of H3 (Santos-Rosa et al. 2009)
3. IGEPAL® CA-630 is a nonionic, non-denaturing detergent. Caution, it is a severe eye irritant.
4. Do not dry excessively the pellet
5. Wear a mask when weighing acrylamide. Unpolymerized acrylamide is a neurotoxin and care should be exercised to avoid skin contact.

## 5. References

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**Figure 1.** Purification of histones visualized in a 16% SDS-PAGE stained with Coomassie blue. The four histones are indicated. \* represents a degradation band.

A

2 $\mu$ l 5 $\mu$ l

70kDa

50kDa

40kDa

35kDa

25kDa

15kDa

10kDa



H3  
H2B  
H2A  
\*  
H4